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Guanine nucleotide-induced Ca^{2+} release in permeabilized murine thymocytes

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GTP and IP_3 induced Ca^{2+} release from an internal store in permeabilized murine thymocytes loaded with Ca^{2+} by ATP. Ca^{2+} release was dependent on the concentration of GTP: half-maximal release with 0.5 μM and maximal release with 10 μM. The GTP effect was completely abolished by 100 μM GTPγS, GMPPNP and UTP. None of the other nucleotides used except ITP induced Ca^{2+} release. When GTP was added after the effect of IP_3 had virtually subsided, and vice versa, further Ca^{2+} release occurred, which led to the conclusion that the mechanism of GTP-mediated Ca^{2+} release may be different from that of IP_3-mediated release.

Ca^{2+} release; GTP; Thymocyte; Inositol trisphosphate

1. INTRODUCTION

Calcium has been of major importance in the study of signal transduction mechanisms [1,2]. In particular, IP_3-induced Ca release from the endoplasmic reticulum has attracted much attention in recent years. On the other hand, in earlier studies, Dawson [3] observed that GTP increased the effectiveness of IP_3 in inducing Ca^{2+} release from rat liver microsomes. Recently, one of the present authors reported that GTP promotes substantial Ca^{2+} release without the addition of exogenous IP_3 in permeabilized N1E-115 cells [4,5]. Using the same cell line, Chueh and Gill [6] reported that IP_3 and GTP function via distinct mechanisms to activate Ca^{2+} release. The same observations were also reported in several other cell systems [7–9]. In the immune systems, Ca^{2+} is also found to be involved in several important cellular activities [10,11]. Stimulation of Ca^{2+} movement in antigenic or mitogenic induction of cell proliferation of lymphocytes was reported by several authors, and IP_3-induced Ca^{2+} release was also recently found in human lymphocytes [12]. However, the mechanism of GTP-mediated Ca^{2+} release in the immune system has not yet been studied. We describe here an investigation of the mechanism of GTP-mediated Ca^{2+} release in permeabilized murine thymocytes, which are known to be precursors of T lymphocytes.

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Abbreviations: IP_3, inositol 1,4,5-trisphosphate; PEG, polyethylene glycol; GMPPNP, guanosine 5′-(β,γ-imido)triphosphate; GTPγS, guanosine 5′-(γ-thio)triphosphate

2. MATERIALS AND METHODS

2.1. *Materials*

All reagents were purchased from Sigma. ^{45}Ca (3.61 Ci/mmol) was obtained from New England Nuclear, mice from Saitama Laboratory Animals and glass fiber filters were the Whatman GF/C type.

2.2. *Preparation of permeabilized cells*

Preparation of permeabilized cells was performed as in [4]. Thymocytes obtained from mouse thymuses were incubated in medium mimicking intracellular ionic conditions and consisting of 140 mM KCl, 10 mM NaCl, 2.5 mM $MgCl_2$ and 10 mM Hepes-KOH at pH 7.0 (designated internal medium), and were

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permeabilized with 0.005% saponin (final) at 37°C for 5 min, followed by washing twice with internal medium. More than 98% of the cells treated by this method were permeable to trypan blue.

2.3. *Ca^{2+} flux measurement*

Permeabilized cells (2–4 × 10^7 cells/ml) were incubated at 37°C in samples of internal medium containing different amounts of EGTA to give the desired free Ca^{2+} concentrations and 3% (w/v) PEG (M_r 6000). Free Ca^{2+} concentration was controlled with EGTA using the stability constants and computer program described by Fabiato and Fabiato [13]. Ca^{2+} uptake and Ca^{2+} release in permeabilized lymphocytes were followed by withdrawing aliquots of 200 μl from the samples at appropriate time intervals. The reaction mixture was composed of ^{45}Ca at 1.25 μCi/ml and 1 mM ATP in internal medium. After 10 min, Ca^{2+} release from internal stores was induced by the addition of GTP or IP_3. Samples on the filters were immediately washed three times with 3 ml ice-cold internal medium containing 1 mM $LaCl_3$. The radioactivity was counted using a liquid scintillation counter.

3. RESULTS AND DISCUSSION

Ca^{2+} uptake into the intracellular calcium storage sites of permeabilized thymocytes reached a plateau within 10 min after addition of ATP and $^{45}CaCl_2$. These Ca^{2+}-loaded cells were tested for Ca^{2+} release from the store site in the presence of GTP, IP_3 or A23187. As shown in fig.1, rapid release of about 60% of the intracellularly stored

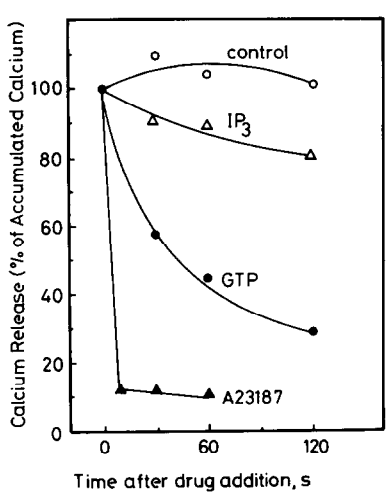

Fig.1. IP_3- and GTP-induced calcium release in permeabilized thymocytes. $[Ca^{2+}]_{free}$ = 150 nM. Ca^{2+} was incorporated into cells (4 × 10^7 cells/ml) for 10 min by 1 mM ATP. Then internal medium (○), 10 μM GTP (●), 5 μM IP_3 (△) or 5 μM A23187 (▲) was added. Ca^{2+} accumulated: 23.8 ± 1.9 pmol/10^6 cells.

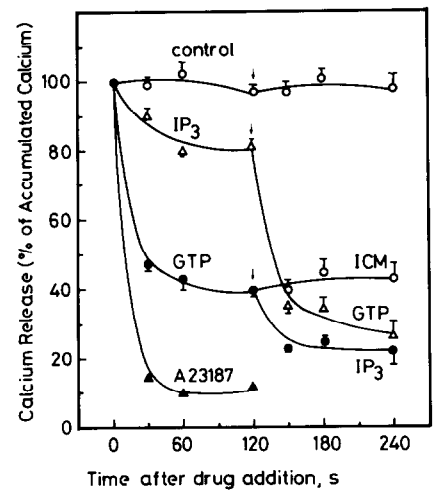

Fig.2. Correlation between IP_3- and GTP-induced Ca^{2+} release. $[Ca^{2+}]_{free}$ = 150 nM. Ca^{2+} was accumulated within the permeabilized cells (4 × 10^7 cells/ml) for 10 min by 1 mM ATP. Then internal medium (○), 10 μM GTP (●) or 5 μM IP_3 (△) was added at zero time. Further internal medium (ICM) (○), or 5 μM IP_3 (●) and 10 μM GTP (△) were added as indicated. (▲) Results on the addition of 5 μM A23187. Ca^{2+} accumulated: 18.4 ± 1.5 pmol/10^6 cells.

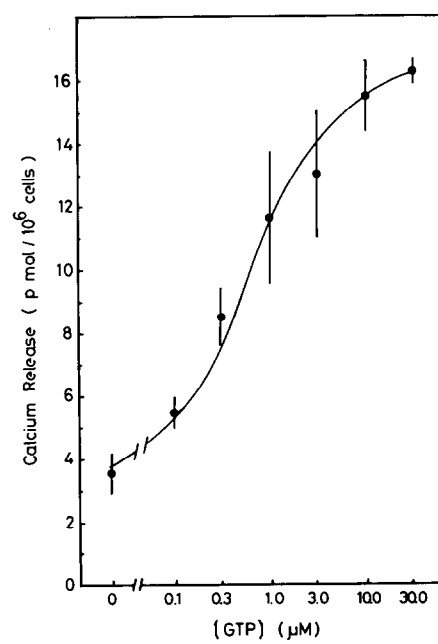

Fig.3. Dose dependency curve of Ca^{2+} release. $[Ca^{2+}]_{free}$ = 150 nM. Calcium was taken up into permeabilized cells (2 × 10^7 cells/ml) for 10 min in the presence of 1 mM ATP. Varying concentrations of GTP were then added. Ca^{2+} release after 1 min was expressed for 10^6 cells.

Ca^{2+} was observed immediately after the addition of GTP. However, only about 15% of the releasable Ca^{2+} was released by IP_3 and maximal release was obtained with 5 μM IP_3 (not shown). The release was not inhibited by oligomycin, antimycin A or ruthenium red, and so the releasable Ca^{2+} was stored in organelles other than the mitochondria. The GTP- and IP_3-sensitive intracellular Ca^{2+}-storage sites in the thymocytes may be in the endoplasmic reticulum, as reported in relation to other cell systems [4–9]. These results were consistent with those of Eberl and Schnell [12].

Chueh and Gill [6] reported that IP_3 and GTP activated Ca^{2+} release from the endoplasmic reticulum of a neuronal cell line via a distinct mechanism. Therefore, we have also investigated the relationship between IP_3- and GTP-mediated Ca^{2+} release processes in permeabilized thymocytes. We first observed the effects of these substances by sequential addition. When IP_3 was added 2 min after Ca^{2+} release initiated by GTP, and vice versa, the total amounts of Ca^{2+} released were almost the same. The release was only additive, and no enhancement by GTP or IP_3 of IP_3- or GTP-mediated release (respectively) was observed (fig.2). These results may indicate that these two ligands mediate Ca^{2+} release via different mechanisms.

Calcium release from permeabilized thymocytes is highly sensitive to GTP. Thus, half-maximal release occurred at 0.5 μM GTP, and maximal release at approx. 10 μM (fig.3).

Among the nucleotides tested, only ITP induced Ca^{2+} release from thymocytes (table 1).

We next examined the effect of several nucleotides and their derivatives on GTP-mediated Ca^{2+} release in thymocytes, the results being summarized in table 1. As shown, 100 μM UTP, 10 and 100 μM GTPγS, and 100 μM GMPPNP inhibited GTP-mediated Ca^{2+} release.

These results indicate that GTP induced Ca^{2+} release in permeabilized murine thymocytes.

Table 1

Effect of nucleotides and their derivatives on Ca^{2+} release

	Concentration (μM)	Ca^{2+} release	Ca^{2+} release with 10 μM GTP
		(% of accumulated Ca^{2+})	
Control		0	–
GTP	10	49	–
UTP	100	3	5
CTP	100	7	43
ITP	100	22	34
TTP	100	0	50
GMP	10	0	–
	100	7	–
GDP	10	5	–
	100	9	–
cGMP	10	10	50
	100	5	–
Guanosine 5′-tetraphosphate	100	10	25
GTPγS	10	12	5
	100	9	5
GMPPNP	100	6	5

Ca^{2+} was incorporated into permeabilized thymocytes in the presence of 1 mM ATP for 15 min. Each concentration of the nucleotides was added and Ca^{2+} release was assessed after 2 min as a percentage of total accumulated calcium. The results in the final column were obtained when 10 μM GTP was added together with the nucleotides

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